Histological and Immunohistochemical Study on the Effects of Astragalus Membranaceus versus Adipose Tissue-Derived Stem Cells in Thioacetamide Induced Liver Fibrosis in Male Albino Rats

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By

Maryham George Loka Yacoub

Assistant lecturer of Histology,

Faculty of Medicine, Fayoum University

Under supervision of

Prof. Dr. Seham Abdel Hamid El Kalawy

Professor of Histology, Faculty of Medicine,

Cairo University

Prof. Dr. Mohamed Salah Elgendy

Professor and head of Histology department, Faculty of Medicine,

Fayoum University

Dr. Abeer Ibraheem Abdallah

Assistant professor of Histology, Faculty of Medicine,

Cairo University

Prof. Dr. Laila Ahmed Rashed

Professor of Biochemistry, Faculty of Medicine,

Cairo University

Histology Department

Faculty of Medicine

Fayoum University

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Summary

The liver is one of the most important organs in the human body as it controls homeostasis of the body by metabolizing lipids, carbohydrates and proteins. Liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen. It occurs in most types of chronic liver diseases. HSCs play a principal role in liver fibrosis. Mesenchymal stem cells (MSCs) have extensively been investigated as potential therapeutic options for the treatment of various degenerative diseases and immune disorders. This is mainly due to their differentiation potential and immunoregulatory properties. They are promising for the treatment of liver fibrosis and cirrhosis. Astragalus Membranaceus is one of the Herbal Chinese medicinal plants that were reported to be able to protect the liver, regulate its function, improve its blood circulation, repair hepatocyte damage and relieve symptoms of liver diseases.

This study aimed at comparing the effect of the traditional Chinese medicinal herb, Astragalus Membranaceus, versus mesenchymal stem cells isolated from the rat adipose tissue on fibrotic rat liver induced by thioacetamide.

Fifty four male albino rats were used in this study and they were divided into three main groups which were further divided into sub groups as follows:

Group I (*control*): Consisted of 18 rats and this group was furtherly subdivided into six subgroups, 3 rats each :

1. **Subgroup Ia:** Each rat was intraperitoneally (IP) injected with 1ml sterile distilled water three times weekly for four weeks.
2. **Subgroup Ib:** Each rat received IP injection of 1ml sterile distilled water three times weekly for eight weeks.

3. **Subgroup Ic:** Rats were prepared as in subgroup Ib then they were given daily oral dose of 1ml saline, via intragastric gavage tube, starting from the beginning of the 5^{th} week (29^{th} day) till the end of the 8^{th} week.
4. **Subgroup Id:** Rats were prepared as in subgroup Ib and at the beginning of the 5^{th} week (29^{th} day), each rat received single IP injection of 1ml PBS.
5. **Subgroup Ie** (***Astragalus-control subgroup***): Each rat was prepared as in subgroup Ib, then received a single daily oral dose (500 mg/kg) of Astragalus dissolved in 1ml of saline by the use of intragastric gavage tube at the beginning of 5^{th} week (29^{th} day) till the end of 8^{th} week.
6. **Subgroup If** (***stem cell-control subgroup***): Each rat was prepared as in subgroup Ib, then received a single IP injection of PKH26 labeled rat AD-MSCs ($3x10^6$ cells/ rat suspended in 1 ml PBS) at the beginning of 5^{th} week (29^{th} day).

- **Group II (*untreated thioacetamide group*):** Consisted of 16 rats. They were subdivided into two subgroups, 8 rats each:

1. **Subgroup IIa:** They received IP injection of 200 mg/Kg of TAA dissolved in 1 ml sterile distilled water for each rat, three times weekly for 4 weeks only then they were sacrificed.
2. **Subgroup IIb:** They received IP injection of 200 mg/Kg of TAA dissolved in 1 ml sterile distilled water for each rat, three times weekly for 8 weeks.

- **Group III (*treated group*):** Consisted of 20 rats. They were subdivided into two subgroups, 10 rats each:

2. **Subgroup IIIa (*Astragalus-treated subgroup):*** The rats were treated as in subgroup IIb. Additionally, each rat received a single daily oral dose (500 mg/kg) of Astragalus dissolved in 1ml of saline /rat by the use of intragastric gavage tube at the beginning of 5^{th} week (29^{th} day) till the end of 8^{th} week.
3. **Subgroup IIIb (*stem cell-treated subgroup*)**: The rats were treated as in subgroup IIb. Additionally, they received single IP injection of PKH26 labeled rat AD-MSCs ($3x10^6$ cells/ rat suspended in 1 ml PBS) at the beginning of 5^{th} week (29^{th} day).

The animals of all subgroups were sacrificed at the end of the 8^{th} week except subgroups Ia and IIa, they were sacrificed at the beginning of 5^{th} week (29^{th} day).

Biochemical analysis of AST, ALT, albumin and procollagen and morphometric assessment of the area% of collagen, α–SMA and NF-κB immunopositive cells were performed. Statistical analysis of biochemical studies and morphometric measurements, using ANOVA test and post Hoc test was done.

In the present study, group I (control group) showed the normal hepatic architecture in which cords of normal hepatocytes radiating from the terminal hepatic venules, forming ill-defined hepatic lobules. Normal portal areas situated at the peripheries of these lobules. Minimal amount of collagen could be detected only around the terminal hepatic venules, and in the portal areas. α–SMA immunoreactions was found only in the media of the terminal hepatic venules, and vessels of portal areas. NF-κB stained sections showed negative immuoreactivity.

Subgroup IIa that received TAA for 4 weeks showed thickened connective tissue septa in Mallory's trichromatic

stained sections with cellular infiltration in between the hepatic lobules. There was disorganization of the lobular architecture, marked congestion of the terminal hepatic venule & branches of portal veins which were dilated. Some hepatocytes appeared having homogenous acidophilic cytoplasm and central rounded vesicular nuclei with prominent nucleoli, while others had dark condensed or dissoluted nuclei. Minimal collagen fibers were detected in the connective tissue septa between the hepatic lobules, around the terminal hepatic venule, branches of the portal vein and blood sinusoids. Positive immunoreaction to α-SMA was noticed around the terminal hepatic venule and branches of the portal vein. Additionally, Positive immunoreactive hepatic stellate cells (HSCs) which appeared as branched, stellate cells with multiple fine processes extended between the hepatic lobules, and in between the hepatocytes. NF-κB immunostaining showed few positive cytoplasmic immunoreaction in the hepatocytes and inflammatory cells around the branch of portal vein with negative immunoreactive hepatocytes and positive inflammatory cells around the terminal hepatic venule.

Subgroup IIb that received TAA for 8 weeks showed thickening of the fibrous septa in between the hepatic lobules with disorganization of the lobular architecture in Mallory's trichromatic stained sections. The terminal hepatic venule & hepatic sinusoids were dilated as well as the branches of the portal vein which were also congested. In zone I some hepatocytes show deeply acidophilic cytoplasm with clumped chromatin and others are apparently normal with acidophilic cytoplasm and vesicular nuclei. Hepatocytes of zone II showed

markedly vacuolated cytoplasm some of them revealed dissoluted nucleus others have shrunken and dark nuclei. Most of hepatocytes of zone III have markedly vacuolated cytoplasm and others have vesicular nuclei or pyknotic nuclei. The hepatic sinusoids are noticed to be lined with endothelial cells and prominent Kupffer cells. Marked increase of the collagen fibers were detected in the interlobular septa, around the terminal hepatic venule, portal tracts and in between the hepatocytes. α-SMA immunoreactive cells (stellate shaped with fine processes) extending between the hepatic lobules, around the terminal hepatic venule and the branches of portal vein in the portal areas. Positive cytoplasmic immunoreaction to NF-κB noticed in almost all hepatocytes and inflammatory cells around terminal hepatic venule, around the branch of portal vein and in the epithelial lining of the bile duct.

Subgroup IIIa ***(Astragalus-treated subgroup)*** showed ill defined lobulation with radiating cords of hepatocytes which were nearly normal (with acidophilic cytoplasm and central rounded vesicular nuclei with prominent nucleoli). Some of the hepatocytes were binucleated. The terminal hepatic venule & portal vein appeared congested and the later was dilated. Minimal inflammatory cell infiltration appeared around the branches of portal vein. Minimal amounts of collagen in the connective tissue septa were noticed between hepatic lobules, around the terminal hepatic venule in the portal areas and in between the hepatocytes. Minimal positive immunoreaction to α-SMA was noticed in the walls of the vessels of portal area and terminal hepatic venule. Positive immunoreaction to NF-κB was detected in the cytoplasm of multiple hepatocytes around the

terminal hepatic venule and around branches of portal vein and negative immunoreaction in the inflammatory cells.

Subgroup IIIb *(Stem cell-treated subgroup)* showed PKH26 labeled MSCs by fluorescent microscopy. It also revealed ill-defined lobulation with radiating cords of hepatocytes which were nearly normal (with acidophilic cytoplasm, central rounded vesicular nuclei and prominent nucleoli). Some hepatocytes were binucleated. There was a slight congestion of the terminal hepatic venules & dilatation of portal vein branches. Minimal amounts of collagen around the terminal hepatic venule, and in the portal areas were detected in Mallory's trichromatic stain. Immunoreaction to α-SMA was only seen in the wall of the terminal hepatic venule and in the branch of the portal vein. Positive cytoplasmic immunoreaction to NF-κB was detected in few of the hepatocytes around terminal hepatic venule and negative in the inflammatory cells and in hepatocytes around the branches of the portal vein.

Subgroup IIa showed significant increase in the serum level of procollagen, mean area % of collagen fibers in Mallory's trichromatic stained sections, mean area % of α-SMA, mean area % of NF-κB, serum levels of AST & ALT and significant decrease in albumin compared to control group. *Subgroup IIb* showed the same results as compared to control and subgroup IIa. *Subgroup IIIa* showed significant decrease in the plasma level of transaminases, procollagen, mean area % of collagen, α-SMA, NF-κB and significant increase in albumin when compared to untreated fibrotic subgroups IIa&IIb. Finally, *subgroup IIIb* showed significant decrease in the plasma level of

AST, ALT, procollagen, mean area % of collagen, α-SMA, NF-κB and significant increase in albumin when compared to subgroups IIa, IIb and IIIa.